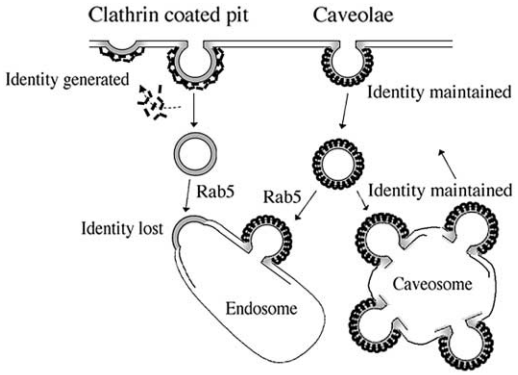

Figure 1. Comparison of Caveolae- and Clathrin-Coated Pit-Mediated Endocytosis

Schematic model comparing caveolae- and clathrin-mediated endocytic processes. Clathrin-coated vesicles interact with early endosomes in a process regulated by Rab5. Caveolae also deliver their contents to the caveosome. In contrast to the clathrin-derived carriers in which the identity of the carriers is generated prior to vesicle formation and lost after delivery to the target membrane, caveolae maintain their identity, forming a stable domain on the endosome or the caveosome from which cargo release can be regulated.

caveosomes run in parallel? Is there communication between the caveosome and early endosome? And what is the role of the caveosome in cellular function? Finally, questions remain regarding the role of caveolin in the caveolae entry pathway. Intriguingly, the study shows a role for caveolin in endosomal sorting but, despite the presumed requirement for caveolin as part of a concentrating device at the cell surface, endocytosis of the two markers studied is not blocked by caveolin downregulation (Nichols, 2002; Pelkmans et al., 2004). If caveolae are absent under these conditions, then a distinct carrier must be involved in the initial internalization step and these carriers must still be able to recruit the machinery required for subsequent targeting to caveosomes and early endosomes. Some of the questions about caveolae have been answered but many more still remain to be tackled before we can understand the formation, function, and dynamics of this enigmatic structure.

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Untangling the Web: Mitochondrial Fission and Apoptosis

Mitochondria exist as an interconnected network that is constantly remodeled by balancing membrane fission and fusion events. A new study by Szabadkai et al. in the October 8th issue of *Molecular Cell* shows that dynamin-related protein 1 (*Drp1*)-induced scission hinders the ability of mitochondria to transport calcium across the cell and mediate apoptosis.

While best known as "cellular powerhouses," mitochondria orchestrate a number of essential functions in addition to energy production in eukaryotic cells, most notably apoptosis and calcium homeostasis. Pro- and antiapoptotic BCL-2 family members act as critical death regulators proximal to mitochondrial dysfunction and irreversible cell damage. During the early stages of apoptosis, a variety of proapoptotic factors (e.g., cytochrome *c*, smac/Diablo) that normally reside within the mitochondrial intermembrane space are released into the cytosol, where they bind to specific target proteins, activate caspases, and lead to cell demise (Danial and Korsmeyer, 2004). During this release, mitochondria undergo complex structural changes, including cristae remodeling and extensive fission. The exact mechanism of mitochondrial remodeling during apoptosis is not completely understood, but recent evidence suggests that some of the same proteins controlling mitochondrial fission in healthy cells may be involved.

Mitochondria work in partnership with the endoplasmic reticulum (ER), the main intracellular Ca^{2+} store, to regulate Ca^{2+} signals within the cell. Following agonist-induced release of Ca^{2+} from the ER through the inositol trisphosphate (IP3) receptor, high Ca^{2+} microdomains (estimated to be in the 50–100 μM range) are generated at tight ER-mitochondrial junctions, activating mitochondrial Ca^{2+} uptake via the low-affinity mitochondrial Ca^{2+} uniporter (Rizzuto et al., 1998). Mitochondrial Ca^{2+} uptake regulates the shape and amplitude of cytosolic

Ca^{2+} transients, modulates Ca^{2+}-dependent mitochondrial Krebs cycle enzymes, and controls permeability transition (PT), ascribed to an inner mitochondrial membrane channel that appears to play a role in cell death following selected stimuli.

To accomplish these distinct roles, mitochondria often exist as a largely interconnected membranous network that can allow efficient energy distribution and calcium transport throughout the cell. Moreover, mitochondrial number and morphology are controlled by frequent fission and fusion events in response to the demands of the cell (Karbowski and Youle, 2003). For example, mitochondrial fission is necessary during cell division to ensure equal inheritance of mitochondria by daughter cells.

While the precise molecular mechanisms involved in mitochondrial scission are not entirely understood, genetic studies from yeast, *Drosophila*, and *Caenorhabditis elegans* have revealed that certain dynamin family members are essential for this process. Dynamins are large GTPases that control vesicular traffic and endocytosis, perhaps by acting as molecular motors to actively participate in membrane constriction and fission or as signaling molecules that recruit other active components to the scission site (Bossy-Wetzel et al., 2003). Overexpression of dynamin-related protein 1 (Drp-1) leads to mitochondrial fragmentation in *C. elegans* and mammalian cells (Smirnova et al., 2001). Conversely, expression of a dominant-negative Drp-1 mutant defective in GTP hydrolysis (Drp-1K38A) results in a network of extremely interconnected mitochondria (Labrousse et al., 1999). Drp-1 appears to function as part of a multiprotein complex at the mitochondrial membrane. Fis-1, a 17 kDa transmembrane protein located at the outer mitochondrial membrane, seems to target Drp-1 to mitochondria during the initiation of fission. When overexpressed in mammalian cells, Fis-1 likewise leads to mitochondrial fragmentation (James et al., 2003).

Evidence is accumulating to suggest that mitochondrial fission may play an important role in apoptosis. Youle and colleagues demonstrated that Drp-1 translocates from the cytosol to focal regions of the outer mitochondrial membrane during apoptosis initiated by staurosporine. Moreover, a dominant-negative Drp-1 mutant blocked staurosporine-induced mitochondrial fragmentation, cytochrome *c* release, and cell death (Frank et al., 2001). Martinou and colleagues found that overexpression of Fis-1 not only triggers mitochondrial fission but also cytochrome *c* release and apoptosis (James et al., 2003).

In the October 8th issue of *Molecular Cell*, a report by Szabadkai et al. examines the effects of mitochondrial fission on Ca^{2+} homeostasis and Ca^{2+}-dependent cell death (Szabadkai et al., 2004). In an elegant series of experiments, the authors use quantitative three-dimensional microscopy and high-speed imaging to measure mitochondrial Ca^{2+} uptake and intramitochondrial Ca^{2+} dynamics in HeLa cells overexpressing Drp-1. Expression of Drp-1 resulted in fragmentation of mitochondria into smaller units distributed throughout the cytosol without affecting the total volume of the mitochondrial network. In contrast, Drp-1 expression did not disrupt the ER morphology, which was monitored with ER-targeted GFP. Using fluorescence recovery after photobleaching (FRAP) in cells expressing mitochondrial targeted GFP (mtGFP), diffusion of mtGFP within the mitochondrial matrix was limited when Drp-1 was coexpressed, strongly suggesting that the morphological changes represent disruptions in the functional connectivity of the mitochondrial network.

Ca^{2+} handling in Drp-1-expressing cells was monitored using a series of Ca^{2+} probes specifically targeted to the mitochondria, ER, or cytosol. Interestingly, while the authors found no differences in agonist (histamine)-induced ER Ca^{2+} release in cells expressing Drp-1, peak mitochondrial Ca^{2+} uptake and intramitochondrial Ca^{2+} diffusion was significantly decreased. The authors attempt to rule out the possibility that a decrease in ER-mitochondrial contact points could explain the disruption in mitochondria Ca^{2+} handling, as they find no reduction in mito/ER overlap sites using 3D reconstructed images of cells containing two different organelle-localized probes. However, such overlap may not be truly representative of the functional Ca^{2+} channels known to exist between these two organelles in living cells.

Finally, the authors go on to examine the effects of Drp-1 on Ca^{2+}-dependent cell death in response to C_2-ceramide, which directly induces Ca^{2+} release from ER Ca^{2+} stores and leads to mitochondrial permeability transition and depolarization. Consistent with the decreased mitochondrial Ca^{2+} uptake following agonist-induced ER Ca^{2+} release in cells with fragmented mitochondria, Drp-1 partially protected the cells from mitochondrial Ca^{2+} overload and apoptosis in response to C_2-ceramide. In agreement with previously published data, they found that Drp-1 sensitized cells to Ca^{2+}-independent apoptosis in response to staurosporine. Overall, these results strongly suggest that an imbalance between fission and fusion can greatly disrupt the ability of mitochondria to properly distribute Ca^{2+} throughout the cell and regulate apoptosis.

These findings differ somewhat from those reported earlier by Demaurex and colleagues on Ca^{2+} homeostasis during mitochondrial fission induced by Fis-1 (Frieden et al., 2004). In that study, when Fis-1 was overexpressed in HeLa cells, mitochondria fragmented within 4 hr and relocalized away from the plasma membrane into punctuate organelles clustered around the nucleus. This pattern was quite different from the fragmented but randomly dispersed mitochondria seen in response to Drp-1 as described earlier. Using a Ca^{2+}-sensitive pericam probe targeted to mitochondria, the authors reported normal Ca^{2+} uptake in Fis-1 fragmented mitochondria following histamine-mediated Ca^{2+} release from the ER. However, Fis-1 expression interfered with the propagation of intramitochondrial Ca^{2+} signals following Ca^{2+} uptake from the plasma membrane.

If the function of Fis-1 were simply to recruit Drp-1 to the outer mitochondrial membrane at the initiation of fission, we might expect that overexpression of either protein would have a similar phenotype. However, the observed differences may result from varying levels of expression of Fis-1 or Drp-1 in these studies. Alternatively, Fis-1 may have a Drp-1-independent role in maintaining mitochondrial morphology or could serve as an

anchoring protein for other players in mitochondrial fission and fusion. In any case, the study by Szabadkai et al. opens a fascinating new avenue into understanding how mitochondrial morphology influences the ability of this complex organelle to handle Ca^{2+} and induce apoptosis.

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Ena/VASP Family: New Partners, Bigger Enigma

Linking external signals to remodeling of the cytoskeleton is essential to multiple processes during animal development and physiology. Two new studies have uncovered a new family of proteins that can regulate actin dynamics both locally at the membrane interface and globally throughout the cell.

Control of cell adhesion, polarity, and motile behavior is essential to most processes during life, from embryonic development and cell differentiation to immunity and normal physiology. The actin cytoskeleton represents an essential part of the molecular machinery associated with these processes, providing the force for cell movement and for the structural changes needed for cell shape modulation, as well as supplying the intracellular anchoring support for adhesion. However, while the molecular basis of how actin polymerization generates the force to reshape cells and push membranes has now been well characterized (Pollard and Borisy, 2003), the precise mechanisms by which local membrane signaling events can modulate actin dynamics in cells remain largely elusive. Two laboratories working on different systems report in this issue of *Developmental Cell* how they coincidently stumbled upon a new family of adaptor molecules that just might provide that special link between local signaling at the membrane and cytoskeletal remodeling (Krause et al., 2004; Lafuente et al., 2004). The new MRL (for Mig-10/RIAM/Lamellipodin) proteins share conserved RA-like (Ras association), SH3 binding, and PH (phospholipid binding) domains, as well as various proline-rich motifs that confer binding to the G-actin binding protein profilin and binding sites for EVH1 (Ena/VASP homology domain 1, a conserved domain present in proteins of the Ena/VASP family). This combination of protein binding interactions suggests that MRL proteins are potentially capable of directly linking the actin cytoskeleton machinery, through Ena/VASP proteins and profilin, to lipid-based membrane targeting and small GTPase signaling.

Proteins of the Ena/VASP family are actin-associated proteins involved in a range of processes dependent on cytoskeleton remodeling and cell polarity, such as axon guidance, T cell polarization, intracellular pathogen mobility, and lamellipodial and filopodial dynamics in migrating cells (Krause et al., 2003). They share a conserved domain structure comprising an N-terminal EVH1 domain, a central polyproline region, and a C-terminal Ena/VASP homology 2 (EVH2) domain. The EVH1 domain recruits Ena/VASP proteins to receptor signaling/complex such as focal adhesions, while the EVH2 domain mediates tetramerization of Ena/VASP proteins and can bind both monomeric (G) and polymerized (F) actin. The central proline-rich region has binding sites for several SH3 and WW domain-containing proteins, as well as for profilin. Ena/VASP proteins appear to regulate fibroblast motility by modulating the structure of the actin network at the leading edge of lamellipods, through alteration of local actin dynamics (Bear et al., 2002). However, the details of the molecular interactions involved have yet to be fully uncovered, and the mechanism by which Ena/VASP proteins are targeted to the leading edge were still unclear. In the accompanying report, Krause et al. now describe how they have found a suitable candidate for that last function in the form of Lamellipodin (Lpd), a novel EVH1 ligand of Ena/VASP proteins and member of the new MRL family of adaptor proteins (Krause et al., 2004). Lpd binds to Ena/VASP proteins and targets them to the leading edge, presumably via simultaneous binding to $PI(3,4)P_2$, a phospholipid signal associated with polarization during chemotaxis (Servant et al., 2000). Interestingly, Lpd is also recruited at the interface between the membrane and the tail of EPEC and *Vaccinia* virus, two pathogens that